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A Device and Method for Mixing Magnetic Particles with a Fluid

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This invention relates to the use of magnetic or magnetizable particles, and, in particular, to methods of mixing magnetic or (super) paramagnetic particles efficiently with a fluid and the separation of the magnetic particles from a fluid, optionally followed by resuspension of the particles in another fluid.
The invention further provided a device for doing the same.

Magnetic particles are often used in separation processes. There are many biological assay methods and purification methods in which magnetic particles are used. For example, immuno assay methods, nucleic acid hybridization assays and the like. Magnetic particles can also be used in purification methods, to isolate particular components, proteins, nucleic acids, from the material in which they were contained. The particles can be used to separate certain components from a mixture, for example, because they are coated with a reagent with a specific affinity for the component. Magnetic particles can be drawn to, for example, the wall of a container in which the fluid with the magnetic particles was contained and the fluid can be removed and, optionally, be replaced with another fluid. Thus, the particles can be mixed with the fluid from which the specific component is to be removed, the component will bind to the magnetic particle, and a magnet can be used to separate the particles with the component from the remainder of the mixture in the fluid. Optionally the magnetic particles can be washed, and can be separated in another fluid. Or the component can be removed from the particles again into another fluid.

The use of magnetic particles for purifying a nucleic acid (NA) target from a biological sample is well known.
Purification methods for nucleic acid using magnetic particles have for example been described in EP757106 (Toyobo) and WO 96/41811 (Boehringer Mannheim). In these applications methods are described wherein a sample solution containing nucleic acid is treated with a chaotropic substance to release the nucleic acid. After releasing the NA from the biological entity in the lysis buffer, the NA is bound to the magnetic particles. Both particles coated with a target-specific probe as well as particles having a metal oxide coating (e.g. silica), giving a generic binding of all NA contained in the sample are used for this purpose. After binding the target, interfering components such as cell debris, enzymes, proteins anti-coagulants and salt are removed by washing the magnetic particles in a (set of) wash buffer(s). Finally, the purified NA is released from the particles by mixing the particles in a small volume of elution buffer. This process is called elution since it is the nucleic acid that is eluted from th particles.

For efficient washing and elution the magnetic particles need to be well dispersed and mixed in the relevant buffers. In general, this washing and elution process may be

hampered by the aggregation or clogging of th magnetic particles eith r caused by the adsorption on the magnetic particles of specific components in the lysed sample (e.g. g nomic DNA) or by residual magnetic dipole fields induced in the particles. In particular, the use of silica coated (magnetic) particles with samples that contain significant amounts of genomic DNA (whole blood, sputum, tissue), results in a tight pellet that is difficult to process.

Well-known methods for mixing (magnetic) beads in a liquid buffer are vortexing, sonification or pipetting. These methods however are difficult to automate, and/or give risk of sample to sample contamination by aerosol generation or they may degrade the NA target. Furthermore, these methods are not well suited for very small volumes of liquid (typically 0.01ml) as may be required for the elution process.

The method and device according to the invention are especially suitable for use with isolation procedures, where, usually an ingredient is to be isolated in rather pure form from a relatively large volume of sample fluid, and concentrated into a smaller volume of another fluid to be suitable for further use.

In the case of a method for the isolation of nucleic acid such further use may be a nucleic acid amplification method or an assay for the detection of nucleic acid or both.

A method and apparatus for separating and resuspending superparamagnetic particles is disclosed in WO 91/09308 (Diatec instruments).

In this application it was disclosed that superparamagnetic particles may be aggregated and resuspended by subsequent application of different magnetic fields. First and second applications of the magnetic field could be provided with the same magnet, which was then rotated around the container containing the particles to a different location. Two spaced opposed electromagnets, however, could also be used. These electromagnets were energized alternately to produce the first and second magnetic fields that keep the particles in suspension and mix them with the fluid in which they were contained.

A method for the separation of magnetic particles from a fluid is disclosed in US 3985649. The particles may be separated from a fluid by bringing the particles into close proximity with a magnet and moved trough the liquid along the wall of a container. They may even be moved out of the liquid in this way and can be transported to a second container.

In US4988618 a device is described for use with assays wherein multiple small volume samples are tested at the same time. These type of assay can be performed in, for example, microtiter plates. Magnetic microparticles are present in each well of the microtiter plate. The device thus has multiple orifices and the orifices are each surrounded by multiple permanent magnets, preferably four. The resulting structure of magnets and orifices is rigid; the magnets are not intended to be moved and are mounted in fixed relations with respect to themselves and to the base of the device. All magnets are aligned and the field orientation of the magnets may be such that all magnets have the same field direction or neighboring magnets have opposite field directions. The magnet orientation thus results in four spot attraction sites per orifice. The magnets are purely

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meant for separation purposes. It is disclosed in the patent that the device may further comprise means or agitating the reagents within the containers.

The pr sent invention relates to a method and device, which allows efficient mixing of magnetic or magnetizable particles in a fluid, and optionally separation of the particles from said fluid. Use is made of magnetic field of opposite and changing directions. It has been found that, when magnetic or magnetizable particles in a fluid are subjected to these magnetic fields, the particles are, under the influence of the field, efficiently contacted with the fluid. Such particles normally may tend to form a clot, which can prevent efficient mixing with a fluid. It has been found that, by subjecting the container in which the fluid and the particles are comprised, to magnetic fields of different and changing directions, the particles are efficiently separated from each other and drawn trough the fluid in such a way that a very efficient mixing process occurs. The method allows efficient mixing of particles with even very small fluid volumes. The method of the invention therefore has the advantage that it may save in, for example, washing fluids and may allow the reduction of the volume of fluid needed. Thus, for example in isolation procedures, the method of the invention allows the purification of reagents in high concentrations. Beside, whereas prior art methods can be laborious and time consuming, the method is fast and easy to perform.

Thus, provided with the invention is a method of mixing, in one or more container(s), magnetic or (super)paramagnetic particles with a fluid, using more than one magnets, whereby the containers are subjected to magnetic fields with different and changing directions by moving the magnets with respect to the position of the container(s) and/or by moving the containers with respect to the positions of the magnets.

With "mixing" in this context is meant that the particles and the fluid are brought in close contact. Mixing thus, means "contacting" in a very efficient manner, such as when particles would be washed or reacted with components present in the fluid. Mixing, in this context, does not necessarily provide a homogeneous mixture after the process is finished. The particles may, when the magnets are removed, segregate to the bottom of the container in which they are comprised or may be held to the wall of the container in a particular location by the magnets. The mixing process can for example be used to wash the particles or to react the particles with a component of the liquid, or to bind a component of the liquid to a reagent coated on the particles. Likewise, the mixing process may result in the elution of a certain component originally present on the particles into the surrounding liquid. The method of the invention is applicable in each of these processes and provides an efficient rapid and convenient way of contacting magnetic or magnetizable particles with a volume of a certain fluid.

The present invention thus provides a generic method for mixing magnetic particles with a fluid almost independent of their level of pelleting/aggregation. The method further allows releasing of reagents bound to the particles, for example nucleic acid, from the particles and concentration into a small volume. The method is easy to automate and well suited for high throughput formats. It minimizes the risk of contamination by droplets or aerosols.

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During a washing (or elution) cycle the (aggregated) particles are dragged through the liquid from left to right by placing a first magnet close to the outside right wall of the vessel and subsequently withdraw this first magnet and simultaneously place a second magnet close to the opposite (left) wall of the vessel in order to drag the particles into the opposite direction. The present invention furthermore provides a device for performing said method.

The device according to the invention comprises means for holding the containers and more than one magnets and means for moving said magnets with respect to the position of said containers and/or means for moving said containers with respect to the position of said magnets in such a way that the containers are subjected to magnetic fields with different and changing directions.

Preferably the magnets are moved with respect to the containers.

The containers may have any convenient shape. Any vessel, suitable for holding a fluid sample in which magnetic particles are dispersed can be used. Preferably the vessels are suitable for holding small liquid samples. For example, they may be Eppendorf cups, PCR containers or micro-titer plate strips).

The magnets may be placed in different geometries with respect to the containers. Any geometry which allows the movement of the magnets with respect to the position of the containers or the other way around, and which will result in magnetic fields of different and changing polarity in each container can be used.

It was found that this washing (or elution) process become particular efficient with the two magnets arranged in such a way that they strongly repel each other (by facing each other with similar poles N-N or S-S). Due to this arrangement the magnetic field lines in the area in the vessel where the magnetic beads are located show a strong and sudden change in direction during each cycle. When the container is placed between two magnets that strongly repel each other because their similar poles are facing each other (N-N or S-S) the slightest movement of either one of the magnets or of the container with respect to each other will result in sudden strong changes of the magnetic field to which the particles in the container are subjected. It has been found that this results in a very efficient way of mixing the particles with the fluid, even when the particles as such tend to form a clot or had already formed a clot within the fluid.

The magnets are preferably arranged in such a way that each magnet repels each of its neighboring magnets.

The magnets may be placed in line in such a way that magnets of opposite polarities can be moved back and forth on straight parallel paths along opposite sites of each container in such a way that the direction of the magnetic field in each container is repeatedly reversed.

This may advantageously be achieved by placing the magnets in line in such a way that all magnets that are in line have th ir poles oriented in the sam direction, and that all magnets in a neighboring line, that is on the other side of th containers next to the first lin of magnets, have their poles oriented in the reverse direction with respect to the poles of the magn ts in th first line.

When the magnets are moved, this may result in the containers being repeatedly placed between two magnets that face each other with the same pole.
The magnets and containers may be placed in parallel rows and the rows of magnets can be moved in opposite directions alongside the rows of containers.
But, of course, based on the basic concept of the method of the invention other geometries can likewise be devised.
The basic concept of an embodiment of a device according to the invention wherein the magnets are movable with respect to the containers is illustrated in Fig.1. The magnetic particles are in a liquid buffer contained in a vessel. The (aggregated) particles are dragged through the liquid from left to right and v.v. by translating a set of at least two magnets arranged such that the magnetic field induced in the vessel changes polarity upon each movement of the magnets.
The method can be used with more containers and magnets. Thus the method and device according to the invention allow for batch-wise processing of several vessels simultaneously. The method and device according to the invention are especially suitable for treating a large number of fluid volumes in each of their respective containers at the same time.
In a preferred embodiment of the device according to the invention the containers and the magnets are placed in intervening array geometries. This layout allows the use of the method of the invention to give a high throughput format.
An embodiment wherein the containers and the magnets are placed in intervening array geometries is illustrated in fig.2. The vessels (e.g. Eppendorf cups, PCR containers or micro-titer plate strips) are placed in an array geometry with the magnets fixed to a second array that translates with respect to the vessels.
In this way a large series of samples is processed simultaneously. Addition and aspiration of liquids may be by hand or by an automated multi-tip dispenser instrument as know in the art.

The method of the invention may also be used with a closed system. That is, a system wherein the liquid, for example, is not contained in a vessel, but in a tube. Thus, with containers, as used with the method of the invention, not only containers used in batch wise processes are meant but also containers used in closed systems, such as tubes and the like. Such an alternative embodiment of a device according to the invention illustrated in figure 3. The particles and liquid are not contained in a vessel but in a tube, allowing processing the particles in a closed system.

Depending on the exact intended use of a device of the present invention several modifications and variations on the above-described theme are possible. For example, the shape of the container may be modified and further modifications as to the location of the magnets with respect to said containers can be made as well.
A device according to the present invention is especially suitable for use with methods for the purification of, for example, nucleic acid from biological starting material.
For a specific purpose th device can be further modified to match the intended use.

The adjustments may result in better ways for separating the particles from the liquid. The device may also be adjusted in such a way that it can be us d with different sample fluid volum s.

In a preferred embodiment according to the invention the magnets can not only be moved with respect to the position of the containers but can also be moved in a direction along the walls of the containers (which would be vertical, when the containers are in an upright position).
In this way, the position of the magnets can be adjusted according to the volume of the fluid in the containers. Thus, when there is only a very small fluid volume to be mixed with the particles the magnet will be in a position that is lower than the position it will have when there is a larger volume of fluid in the same container.

The fact that the magnets can be moved in a vertical direction has the additional advantage that the magnets can now also be used to draw the particles to the lower part of the container, even when a bigger fluid volume is used. Thus, this allows the removal of a large part of the fluid volume, for example by a pipettor, while the magnet holds down the particles.
Optionally, the magnets, when they can be moved in a vertical direction along the walls of the containers, can also be used to draw the particles alongside the wall of the container till a position above the surface of the fluid. In that way the particles can be separated from the fluid and the remaining fluid may be removed from the container or, for example, be replaced by another fluid after which the particles may be drawn down below the liquid level and mixed with the new fluid using the magnets.
It is evident that the design of the device allows many variations in the methods of its use and all fall within the scope of the invention.
The use of the movement of the magnets in a vertical direction is illustrated in figure 4.

To allow the use of the device with a procedure involving the subsequent treatment of the particles with several liquids in different volumes and achieve an efficient mixing and separation of the particles with/from the respective fluids, adjustments can be made to the containers as well.
If a large container is used with a very small fluid volume the problem may arise that the particles can no longer be contacted with the fluid, simply because the fluid volume is more or less spread out over the bottom of the container and doesn't even cover the particles.
Thus, containers can be devised that can be used with different liquid volumes and still allow efficient mixing of the fluid volumes with the particles. Such containers and the use thereof are likewise part of the present invention.
To allow the use of fluids of considerable different volume a container can be used that comprises a part that is suitable for containing small fluid samples, while this part is connected to a part that is suitable for containing large volume sampl s. An example of such a container is illustrated in figure 4.

The multi-purpose container as depicted in figure 4 is provided with a tip with a relatively small diameter suitable for containing small volume samples, while the part on top of the tip is suitable for containing larger volume samples.
As indicated in figure 4 this container is suitable for using the device with small and large fluid volumes and the height of the magnets with respect to the container can be adjusted accordingly.
Moreover, the tip allows the collection of the particles from a large volume sample by moving the magnets in the downward orientation. The major part of the liquid can then be removed from the container without accidentally removing any of the particles.

A device according to the invention is especially suitable for use in a method for the isolation of nucleic acid from biological samples.
A typical method for the isolation of nucleic acid is the method as devised by R.Boom et al., as disclosed in EP 389063.
The "Boom method" involves the treatment of the biological material with a lysis buffer containing a chaotropic substance such as guanidine-isothiocyanate and a siliceous solid phase. The siliceous solid phase may be provided in the form of magnetic silica particles. The nucleic acid released from the material by the lysis buffer will adhere to the (magnetic) siliceous particles. Thus, the particles and the biological material in the lysis buffer should be thoroughly contacted with each other, which is where the use of a device according to the method would come in. The particles with the nucleic acid adhered thereto can subsequently be separated from the remainder of the sample using a magnet (which can also be done with a device according to the invention provided that it is adapted for that purpose). Subsequently the nucleic acid containing particles should be washed, which requires the mixing of the particles with a washing buffer. This is another function that may be performed by the device according to the invention. The particles are then removed from the washing liquid and contacted with an elution buffer (again, thorough contact between the particles and the elution buffer is required) and the nucleic acid is thus released from the particles into the elution buffer. In general, liquid volumes required for washing will be about 10 times larger than for elution. A typical volume for washing (per vessel per wash step) is 0.2-0.5 ml. The typical volume for elution buffer is 0.010/0.050ml

The embodiment of the device wherein the magnets can be moved in the vertical direction as well and containers are used that have a tip for the use of smaller liquid volumes is especially suitable for use with the so-called "Boom method" for the isolation of nucleic acid as described above.
When the device would be used with a method like the Boom method this can be performed with the following procedure:
A typical volume required for a washing step would be 0.2 to 0.5 ml, which is a relatively large volume. Therefore, during washing the magnetic particles are in the upper part of the vessel (level 1, fig.4 situation 1). How ver, for most applications the nucleic acid target needs to b concentrated in a buffer volume of typically 10 to 50µl. Such small

liquid volumes are hard to handle. It is difficult to control th size of such a small volum as well as to manipulate it in a vessel in combination with magn tic particl s to form a suspension for performing bound-free steps.

Fig.4 shows a method that overcomes the above difficulties.

After completing the washing procedure the particles are captured at the side of the vessel wall (level 1, situation 1) and the wash liquid is aspirated with a pipetter tip.

Next, the vessel is filled with fresh elution buffer (about 0.2ml) and the magnetic particles are transported down to the lower end of the vessel (level 3) by bringing the magnets down (situation 2). Transport of particles can be accelerated by translating the magnet array as is done during washing as it moves downward. The composition of the ET buffer is such that no nucleic acid is released from the silica as long as the buffer temperature is not above RT.

Next, while aspirating, the tip is introduced into the vessel until its lower end is at a level that corresponds to the required volume of ET buffer (e.g. 10µl, see situation 3).

Next, a heat block is brought into contact with the vessel to heat up the temperature of the buffer to 55-60°C (situation 4)

Next, the actual elution procedure starts by translating the magnets horizontally as during the washing procedure, but now at level 3. Preferably, during elution, the heat block remains in contact with the vessel to keep the temperature of the elution buffer at 55-60°C.

Finally, after completing the elution, the heat block is moved away from the containers (down)l and the magnets are moved up to level 2 (situation 5) to withdraw the particles from the elution buffer that is now ready for further processing (amplification, sequencing,)

Preferably, in order to allow the heat block to contact the vessel during elution without disturbing the elution process (situation 4), the heat block has a special design that accounts for the dimensions of the magnetic array as well as for the shape of the vessel. The heat block preferably is produced from a material that is non-magnetic. For example, the heat block is produced from aluminum and contains a ceramic heater element as is known from the state of the art.

Thus, it is illustrated how the device can be used to automate and speed up existing procedures, that now have to be perform, either by hand or in more complicated automated devices.

Of course, the use of a device according to the invention will find its application in many biological assays or purification processes.

BRIEF DESCRIPTION OF THE FIGURES:

Figure 1: The basic concept of an array according to the invention

Figure 2: Device wherein the holders for the containers and the magnets are placed in intervening array geometries and the magnets are placed in line in such a way that magnets of opposite polarities can be moved back and forth on straight

parallel paths along opposite sites of each container in such a way that the direction of th magnetic field in each container is repeatedly reversed.

Figure 3: Device wherein the containers are part of a closed system, e.g. a tube.

Figure 4: D vice wherein the magnets can also be mov d in a vertical direction so as to be positioned at different heights with respect to the walls of the containers and the containers are tube-shaped vessels provided with a tip for holding small liquid volumes.